Novatia

Title: MS/MS Analysis of a 24-mer DNA Sample
Project: Sequence confirmation by MS/MS
Client: Dr. Jose Customer, OligosRus
Sample ID's: 24-mer
Date: May 29, 2009
Analyst: Mark E. Hail, Ph.D., Novatia, LLC

Summary:

Electrospray ionization (ESI) mass spectrometry (MS) and tandem mass spectrometry (MS/MS) were used to analyze an oligonucleotide sample consisting of a 24-residue DNA oligonucleotide. MS/MS analysis produced nearly a complete set of complimentary fragment ions that are confirmatory for the submitted sample and its putative oligonucleotide sequence. In addition, the exact mass of the intact oligonucleotide was also consistent with expected value of 7399.27 Da.

MS/MS spectra were deisotoped and converted to zero-charge mass spectra (see experimental section). A list of predicted zero-charge fragments is shown in **Table 1**. The fragments denoted as type **b**, **a-B**, and **d-H_2O** contain the 5' terminus, while fragments of type **w, w-H_2O** and **y** contain the 3' terminus[1]. DNA oligonucleotides predominantly produce ions of type **a-B** and **w**, as was observed with this sample. See **Figure 1** for a schematic diagram of mass spectral oligonucleotide fragmentation and nomenclature. As shown by the highlighted fragments in **Table 1** and the supporting spectrum in **Figure 2**, a nearly complete set of complimentary fragment ion data was obtained for this oligonucleotide. There are some "gaps" in the fragment ion coverage, mainly near the 3' side of the **T** nucleotides. Incomplete fragmentation at **T** is common for oligonucleotides. These phenomena are a result of ion chemistry effects, where the localization of charge is not favored at particular sites along the oligonucleotide chain.

The exact (monoisotopic) mass was also obtained for the intatct 24-mer DNA sample as an additional measure of oligonucleotide structural confirmation. The exact mass was determined from the normal ESI/MS spectrum using deisotoping software. The result is shown in Figure 3. The oligonucleotide exact mass was measured to within 0.01 Da (1.6 ppm) of the expected value, which is within the expected measurement error for the high-resolution mass spectrometer that was employed.

[1] James A. McCloskey et al, *Anal. Chem.* 1996, **68**, 1989-1999.

Experimental:

Sample preparation: Samples were diluted to ~10 uM in water containing 10 uM EDTA, 0.1% DIEA, 1% HFIPA. (DIEA = diisopropylethylamine, HFIPA = hexafluoroisopropanol). 1 nmole samples were desalted on a 1 x 10 mm trap column of a Novatia Oligo HTCS system. Samples were eluted and collected in a 1.5 mL Eppendorf tube (~500 uL total volume).

Mass Spectrometry: Thermo LTQ Orbitrap Discovery (Thermo Scientific).

Mode: ESI, Negative ions

Sample introduction: The samples were directly introduced into the ESI ion source at a flow rate of 5 uL/min via the integrated syringe pump on the LTQ-Orbitrap mass spectrometer.

MS^n parameters: resonance excitation with wideband activation, 3 u isolation width, 35 V normalized collision energy, q=0.18, precursor ion: m/z 1232.7 (6^-), data acquired using 30,000 resolution Orbitrap scan with signal averaging of ~1 min or more per spectrum.

Data processing: MS^n spectra were processed with PPL Review (Positive Probability, LTD) to convert the multiply-charged fragment ion mass spectral data to deisotoped, zero-charge mass spectra. The deisotoping algorithm converts each isotope cluster into a zero-charged monoisotopic mass for each oligonucleotide mass or fragment. Software written in-house is used to calculate the expected MS/MS fragments from a given oligonucleotide sequence. These masses are compared with experimental values obtained from the deisotoping algorithm.

Table 1. Predicted zero-charge MS/MS fragments for the 24-mer DNA oligonucleotide sequence: GCA GAA AGC GTC TAG CCA TGG CGT

	5' Fragments				3' Fragments			
n	**5'**	**a-B**	**b**	**d-H2O**	**w**	**w-H2O**	**y**	**3'**
1	G		267.09675	329.0525	322.0566	304.0460	242.0903	T
2	C	427.0893	556.14313	618.0989	651.1091	633.0986	571.1428	G
3	A	716.1357	869.20073	931.1565	940.1555	922.1449	860.1892	C
4	G	1029.1933	1198.25325	1260.2090	1269.2080	1251.1975	1189.2417	G
5	A	1358.2458	1511.31085	1573.2666	1598.2605	1580.2500	1518.2942	G
6	A	1671.3034	1824.36846	1886.3242	1902.3066	1884.2960	1822.3402	T
7	A	1984.3610	2137.42606	2199.3818	2215.3642	2197.3536	2135.3978	A
8	G	2297.4186	2466.47858	2528.4344	2504.4106	2486.4000	2424.4442	C
9	C	2626.4711	2755.52496	2817.4807	2793.4569	2775.4464	2713.4906	C
10	G	2915.5175	3084.57747	3146.5332	3122.5094	3104.4989	3042.5431	G
11	T	3244.5700	3388.62351	3450.5793	3435.5670	3417.5565	3355.6007	A
12	C	3548.6161	3677.66988	3739.6257	3739.6131	3721.6025	3659.6468	T
13	T	3837.6624	3981.71592	4043.6717	4028.6595	4010.6489	3948.6931	C
14	A	4141.7085	4294.77353	4356.7293	4332.7055	4314.6949	4252.7392	T
15	G	4454.7661	4623.82604	4685.7818	4661.7580	4643.7474	4581.7917	G
16	C	4783.8186	4912.87242	4974.8282	4950.8044	4932.7938	4870.8381	C
17	C	5072.8650	5201.91879	5263.8746	5279.8569	5261.8463	5199.8906	G
18	A	5361.9113	5514.97639	5576.9322	5592.9145	5574.9039	5512.9482	A
19	T	5674.9689	5819.02243	5880.9782	5905.9721	5887.9615	5826.0058	A
20	G	5979.0150	6148.07495	6210.0307	6219.0297	6201.0192	6139.0634	A
21	G	6308.0675	6477.12747	6539.0832	6548.0822	6530.0717	6468.1159	G
22	C	6637.1200	6766.17384	6828.1296	6861.1398	6843.1293	6781.1735	A
23	G	6926.1664	7095.22636	7157.1821	7150.1862	7132.1756	7070.2199	C
24	**T**							**G**

Note: Fragments highlighted in green were detected in the MS/MS data. Ion types are depicted in Figure 1.

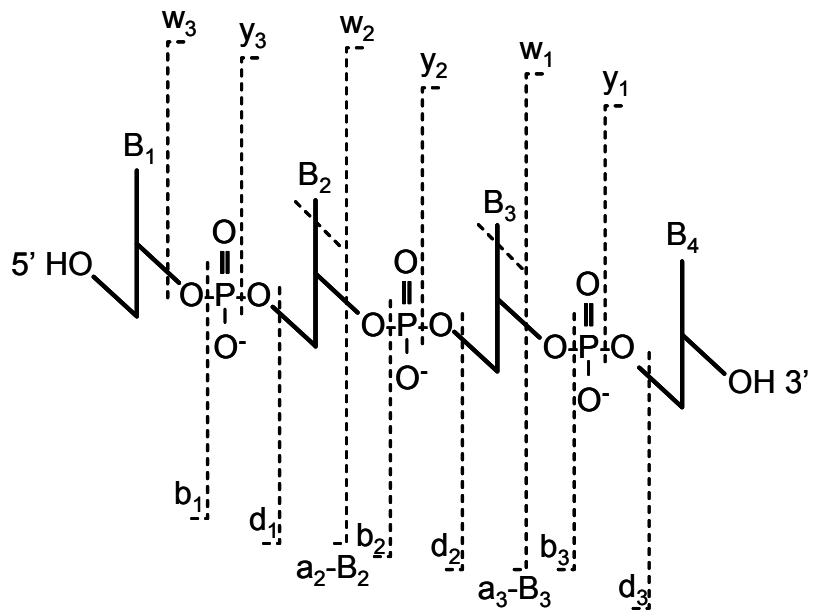

Figure 1. Schematic of oligonucleotide mass spectral fragmentation. Oligonucleotides fragment along the phosphate backbone producing a set of ions containing the 5' terminus (**a-B**, **b**, and **d**) and another set of ions containing the 3' terminus (**w** and **y**). Losses of H_2O from these ion types are also common with **d** and **w** ions. The **a-B** ion is somewhat unique in that it involves base loss in addition to cleavage at the phosphate.

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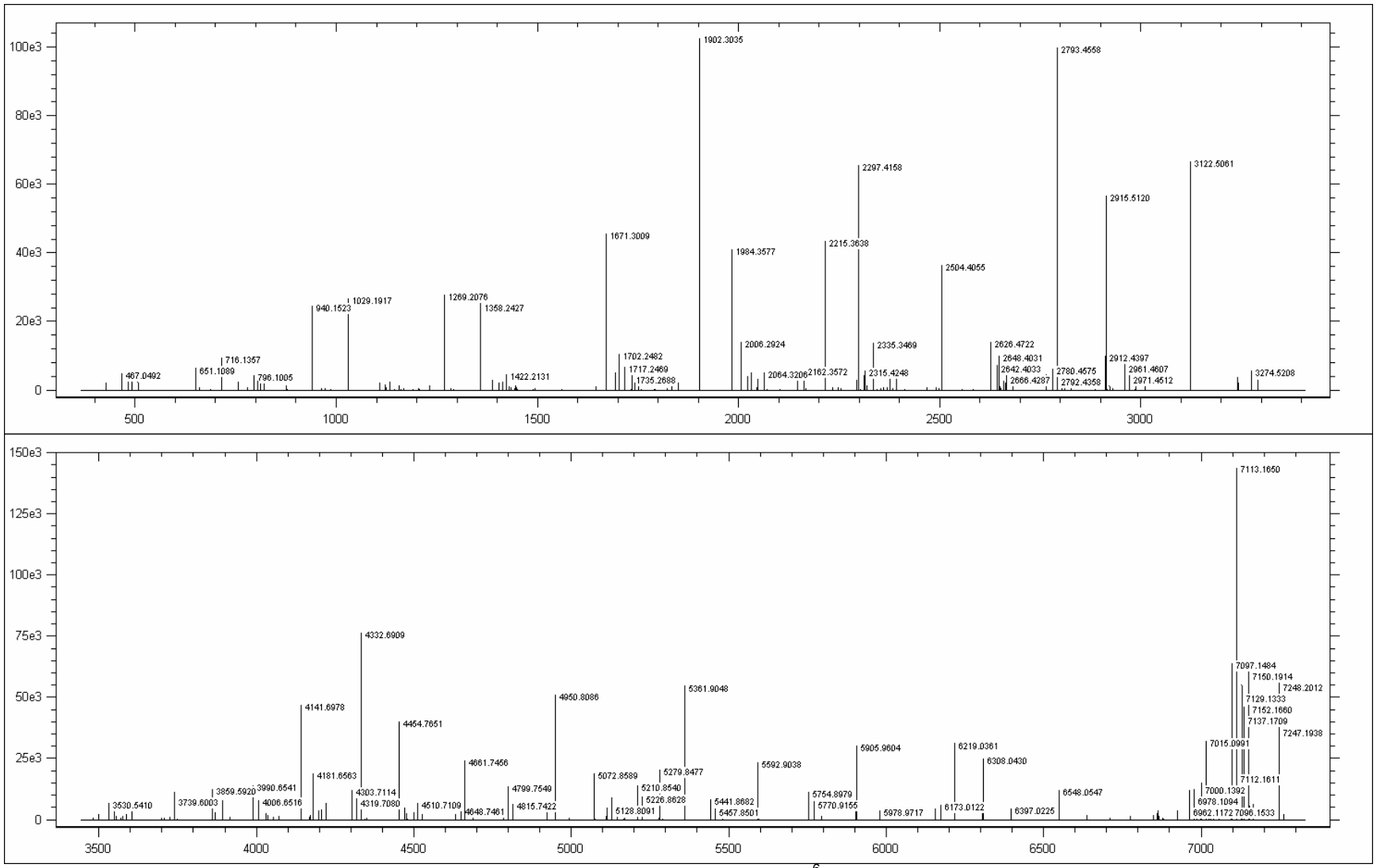

Figure 2. Deisotoped zero-charge MS/MS product ion spectrum of m/z 1232.7 $[M-6H]^{6-}$ from 24-mer DNA sample. The fragments produced are highlighted in green in **Table 1**.

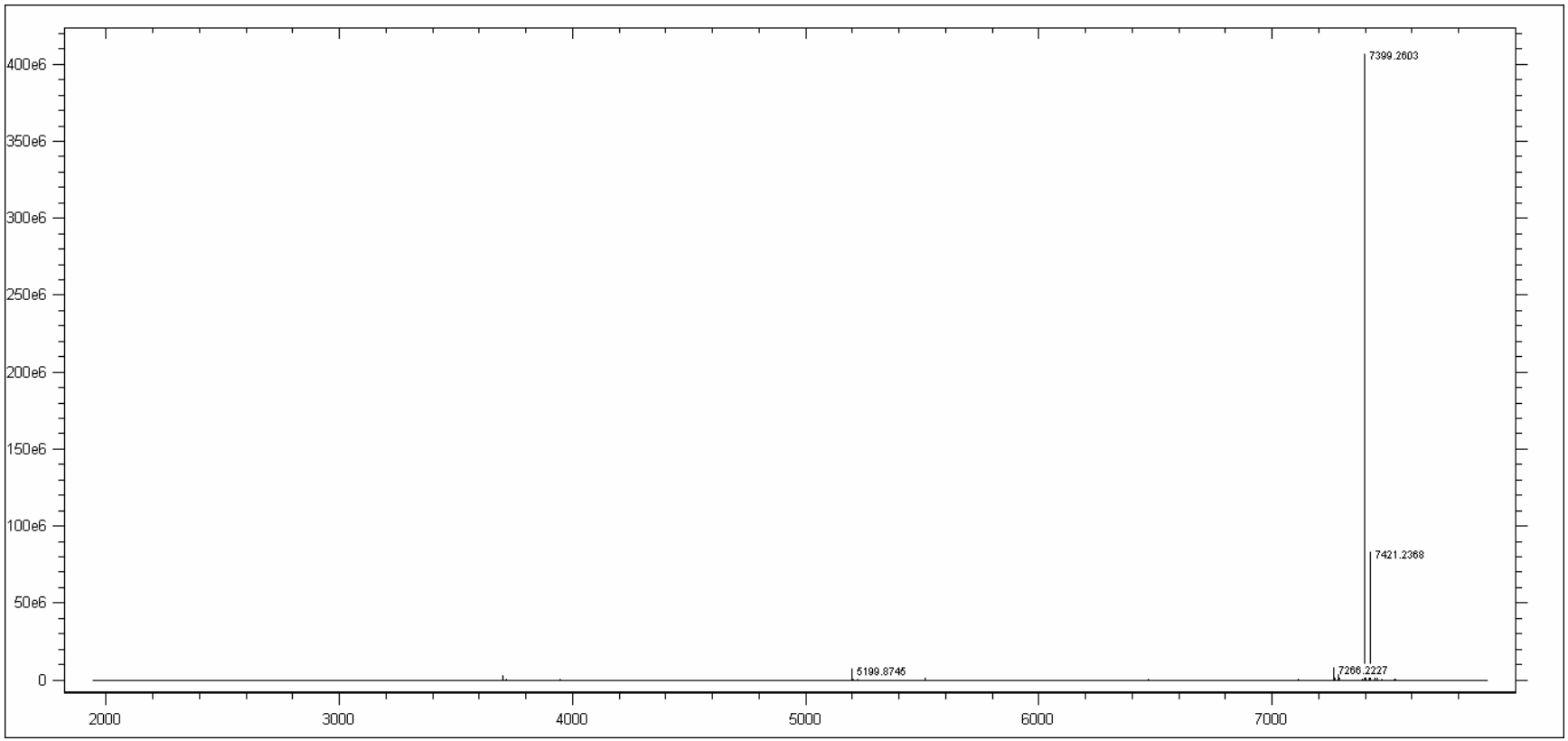

Figure 3. Deisotoped ESI spectrum of 24-mer DNA sample. The expected monoisotopic mass of the sample is 7399.2724. The observed mass of 7399.26 is in agreement within 1.6 ppm.